Phase 2b Controlled Trial of M72/AS01E Vaccine to Prevent Tuberculosis

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Abstract: BACKGROUND: A vaccine to interrupt the transmission of tuberculosis is needed. METHODS: We conducted a randomized, double-blind, placebo-controlled, phase 2b trial of the M72/AS01E tuberculosis vaccine in Kenya, South Africa, and Zambia. Human immunodeficiency virus (HIV)-negative adults 18 to 50 years of age with latent M. tuberculosis infection (by interferon- release assay) were randomly assigned (in a 1:1 ratio) to receive two doses of either M72/AS01E or placebo intramuscularly 1 month apart. Most participants had previously received the bacille Calmette-Guérin vaccine. We assessed the safety of M72/AS01E and its efficacy against progression to bacteriologically confirmed active pulmonary tuberculosis disease. Clinical suspicion of tuberculosis was confirmed with sputum by means of a polymerase-chain-reaction test, mycobacterial culture, or both. RESULTS: We report the primary analysis (conducted after a mean of 2.3 years of follow-up) of the ongoing trial. A total of 1786 participants received M72/AS01E and 1787 received placebo, and 1623 and 1660 participants in the respective groups were included in the according-to-protocol efficacy cohort. A total of 10 participants in the M72/AS01E group met the primary case definition (bacteriologically confirmed active pulmonary tuberculosis, with confirmation before treatment), as compared with 22 participants in the placebo group (incidence, 0.3 cases vs. 0.6 cases per 100 person-years). The vaccine efficacy was 54.0% (90% confidence interval [CI], 13.9 to 75.4; 95% CI, 2.9 to 78.2; P=0.04). Results for the total vaccinated efficacy cohort were similar (vaccine efficacy, 57.0%; 90% CI, 19.9 to 76.9; 95% CI, 9.7 to 79.5; P=0.03). There were more unsolicited reports of adverse events in the M72/AS01E group (67.4%) than in the placebo group (45.4%) within 30 days after injection, with the difference attributed mainly to injection-site reactions and influenza-like symptoms. Serious adverse events, potential immune-mediated diseases, and deaths occurred with similar frequencies in the two groups. CONCLUSIONS: M72/AS01E provided 54.0% protection for M. tuberculosis-infected adults against active pulmonary tuberculosis disease, without evident safety concerns. (Funded by GlaxoSmithKline Biologicals and Aeras; ClinicalTrials.gov number, NCT01755598 .).

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ORIGINAL ARTICLE

Phase 2b Controlled Trial of M72/AS01$_E$ Vaccine to Prevent Tuberculosis

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ABSTRACT

BACKGROUND

A vaccine to interrupt the transmission of tuberculosis is needed.

METHODS

We conducted a randomized, double-blind, placebo-controlled, phase 2b trial of the M72/AS01$_E$ tuberculosis vaccine in Kenya, South Africa, and Zambia. Human immunodeficiency virus (HIV)–negative adults 18 to 50 years of age with latent *M. tuberculosis* infection (by interferon-γ release assay) were randomly assigned (in a 1:1 ratio) to receive two doses of either M72/AS01$_E$ or placebo intramuscularly 1 month apart. Most participants had previously received the bacille Calmette–Guérin vaccine. We assessed the safety of M72/AS01$_E$ and its efficacy against progression to bacteriologically confirmed active pulmonary tuberculosis disease. Clinical suspicion of tuberculosis was confirmed with sputum by means of a polymerase-chain-reaction test, mycobacterial culture, or both.

RESULTS

We report the primary analysis (conducted after a mean of 2.3 years of follow-up) of the ongoing trial. A total of 1786 participants received M72/AS01$_E$ and 1787 received placebo, and 1623 and 1660 participants in the respective groups were included in the according-to-protocol efficacy cohort. A total of 10 participants in the M72/AS01$_E$ group met the primary case definition (bacteriologically confirmed active pulmonary tuberculosis, with confirmation before treatment), as compared with 22 participants in the placebo group (incidence, 0.3 cases vs. 0.6 cases per 100 person-years). The vaccine efficacy was 54.0% (90% confidence interval [CI], 13.9 to 75.4; 95% CI, 2.9 to 78.2; P=0.04). Results for the total vaccinated efficacy cohort were similar (vaccine efficacy, 57.0%; 90% CI, 19.9 to 76.9; 95% CI, 9.7 to 79.5; P=0.03). There were more unsolicited reports of adverse events in the M72/AS01$_E$ group (67.4%) than in the placebo group (45.4%) within 30 days after injection, with the difference attributed mainly to injection-site reactions and influenza-like symptoms. Serious adverse events, potential immune-mediated diseases, and deaths occurred with similar frequencies in the two groups.

CONCLUSIONS

M72/AS01$_E$ provided 54.0% protection for *M. tuberculosis*–infected adults against active pulmonary tuberculosis disease, without evident safety concerns. (Funded by GlaxoSmithKline Biologicals and Aeras; ClinicalTrials.gov number, NCT01755598.)

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One quarter of the global population is estimated to be infected with *Mycobacterium tuberculosis*, and tuberculosis is the leading infectious cause of death worldwide.[1,2] There were an estimated 10.4 million new cases of tuberculosis and 1.7 million deaths from the disease in 2016. An effective tuberculosis vaccine for *M. tuberculosis*–infected persons could have a marked effect on tuberculosis control, including drug-resistant tuberculosis, through interruption of transmission.[3,4] Modeling suggests that the most effective contribution to tuberculosis control would be a vaccine preventing pulmonary tuberculosis in adolescents and young adults.[4] The only licensed tuberculosis vaccine, BCG (bacille Calmette–Guérin), does not offer substantial protection against pulmonary tuberculosis in *M. tuberculosis*–infected adults.[5]

The M72/AS01$_E$ (GlaxoSmithKline) candidate vaccine contains the M72 recombinant fusion protein derived from two immunogenic *M. tuberculosis* antigens (Mtb32A and Mtb39A), combined with the AS01 adjuvant system, which is also a component of the malaria vaccine (RTS,S/AS01, GlaxoSmithKline) and recombinant zoster vaccine (Shingrix, GlaxoSmithKline). The Mtb39A and Mtb32A components of the recombinant antigen elicited specific lymphoproliferation, interferon-γ production, or both in persons with latent and active tuberculosis.[6-8] In phase 2 studies, M72/AS01$_E$ showed a clinically acceptable safety profile and induced humoral and cell-mediated immune responses in healthy and human immunodeficiency virus (HIV)–infected persons, *M. tuberculosis*–infected adults and adolescents, and BCG-vaccinated infants (Table S1 in the Supplementary Appendix, available with full text of this article at NEJM.org).[9-16]

Overall, nonclinical evaluations (antigen-selection approach and in vivo preclinical data) and clinical safety and immunogenicity evidence, based on the ability of the candidate vaccine to induce type 1 helper T cell–type responses, supported a proof-of-concept human trial, despite caveats associated with the available studies in animals.[6-9,17-22] We conducted a proof-of-concept phase 2b trial to evaluate M72/AS01$_E$ in preventing bacteriologically confirmed pulmonary tuberculosis in HIV-negative adults with *M. tuberculosis* infection, defined by a positive interferon-γ release assay. This population was selected on the basis of a higher incidence of pulmonary tuberculosis among persons with a positive interferon-γ release assay than among those with a negative assay, which allowed a smaller sample for proof of concept.[23]

METHODS

TRIAL DESIGN AND OVERSIGHT

The trial is a multicenter, double-blind, randomized, placebo-controlled trial conducted in three African countries in which tuberculosis is endemic (Kenya, South Africa, and Zambia). The randomization was not stratified but was performed with the use of a minimization algorithm that accounted for sex and center (for details, see the Supplementary Appendix). Eleven trial sites were selected on the basis of the local prevalence of tuberculosis and an ability to perform the trial according to Good Clinical Practice guidelines. The QuantiFERON-TB Gold In-Tube assay (QFT, Qiagen) was used at the manufacturer's recommended cutoff point to identify latent *M. tuberculosis* infection. The trial population is being followed up for 3 years after administration of M72/AS01$_E$ or placebo. A prespecified primary analysis was performed when all the participants had completed at least 2 years of follow-up. Immunogenicity and reactogenicity were assessed in a subgroup of 300 participants. The final analysis after 3 years of follow-up and secondary trial objectives, including cell-mediated immune responses, are not reported here because these data are not yet mature.

The trial was undertaken in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The protocol (available at NEJM.org) was approved by ethics committees and regulatory authorities in each participating country. The trial was funded by GlaxoSmithKline Biologicals (trial sponsor) and Aeras. Authors who are employees of GlaxoSmithKline and Aeras were involved in the conception and design of the trial and the collection, analysis, and interpretation of data, and some of them were part of the core writing team (see the Supplementary Appendix for a list of authors' contributions). All the authors vouch for the completeness and accuracy of the data and analyses presented and for the adherence of the trial to the protocol. All the authors reviewed and approved the manuscript before it was submitted for publication. All the participants provided written or witnessed oral informed consent.

Unblinded safety data were reviewed by an independent data monitoring committee. Only the

Table 1. Case Definitions of Tuberculosis (TB).*

Case Definition	Clinical Suspicion†	Culture and PCR Results	HIV Status	Other Condition
First definition (primary end point): definite pulmonary TB disease not associated with HIV infection	Yes	Either test or both tests positive‡	Negative	Sputum collected before initiation of TB treatment
Definition used for the sensitivity analysis of the primary end point: definite pulmonary TB disease (any two positive sputum tests) not associated with HIV infection	Yes	Any two tests positive§	Negative	Sputum collected before initiation of TB treatment
Second definition: definite PCR-positive pulmonary TB disease not associated with HIV infection	Yes	Positive PCR assay and any result on culture‡	Negative	Sputum collected before initiation of TB treatment
Third definition: definite pulmonary TB, not associated with HIV infection	Yes	Either test or both tests positive‡	Negative	Sputum collected up to 4 wk after initiation of TB treatment
Fourth definition: definite pulmonary TB	Yes	Either test or both tests positive‡	Any	Sputum collected up to 4 wk after initiation of TB treatment
Fifth definition: clinical TB (any location)	—¶	—¶	Any	Clinician has diagnosed TB disease and has decided to treat patient
Modified fifth definition: clinical TB (any location) not associated with HIV infection	—¶	—¶	Negative	Clinician has diagnosed TB disease and has decided to treat patient

* Possible deaths due to TB have not been included in any of the case definitions unless the case-definition criteria as stated were met. HIV denotes human immunodeficiency virus, and PCR polymerase chain reaction.
† The participant presented with one or more of the following: cough for more than 1 or 2 weeks, fever for more than 1 week, night sweats, weight loss, pleuritic chest pain, hemoptysis, fatigue, or shortness of breath on exertion.
‡ Results are for any of the three sputum samples collected because of clinical suspicion.
§ Any two tests positive indicates at least two positive cultures, two positive PCR assays, or one positive culture and one positive PCR assay among all test results obtained from the three sputum samples collected because of clinical suspicion.
¶ This is not a mandatory part of the case definition.

external statisticians and the members of the independent data monitoring committee are aware of the trial-group assignments at the level of individual participant data. Anonymized individual participant data and study documents can be requested for further research (see the data sharing statement, available at NEJM.org).

POPULATION

Adults 18 to 50 years of age were eligible if they were healthy or had stable chronic medical conditions, were HIV-negative, had no symptoms of tuberculosis, were QFT-positive, and had a sputum sample negative for *M. tuberculosis* at baseline on a polymerase-chain-reaction (PCR) assay (GeneXpert MTB/RIF, Cepheid). Information on the eligibility criteria and screening procedures is provided in the Supplementary Appendix.

VACCINATION

Participants were randomly assigned to M72/AS01$_E$ or placebo in a 1:1 ratio. Two doses of M72/AS01$_E$ or placebo were administered intramuscularly (0.5 ml) into the deltoid 1 month apart. Information on vaccine and placebo composition is provided in the Supplementary Appendix.

EFFICACY END POINTS

The primary objective of the trial was to evaluate the efficacy of M72/AS01$_E$ to prevent active pulmonary tuberculosis according to the first case definition (primary end point; see Table 1 for case definitions). Secondary trial objectives were vaccine efficacy according to additional case definitions, as well as the immunogenicity, safety, and reactogenicity of the vaccine.

EVALUATION OF SAFETY AND REACTOGENICITY

Serious adverse events, potential immune-mediated diseases, and pregnancies were recorded until 6 months after the second dose. Serious adverse events that were considered by the site investigators to be related to the trial regimen were recorded until the end of the trial. Unsolicited reports of adverse events were recorded for 30 days after each dose. Local and systemic symptoms

were solicited from the immunogenicity subgroup with the use of diary cards for 7 days after each dose. Laboratory testing for clinical chemical and hematologic analyses was performed in the subgroup on days 0, 7, 30, and 37. (For more on safety monitoring, see the Supplementary Appendix.)

EVALUATION OF IMMUNOGENICITY

Blood samples were collected from the immunogenicity subgroup before dose 1, at 1 month after dose 2, and annually until year 3. Anti-M72 IgG antibodies were measured with the use of enzyme-linked immunosorbent assay (ELISA), as described previously (cutoff, 2.8 ELISA units per milliliter).[13]

TUBERCULOSIS SURVEILLANCE

Surveillance of tuberculosis involved both active methods (visits, telephone calls, and text messages) and passive methods (patient reports). Participants with clinical suspicion of pulmonary tuberculosis provided three sputum samples, which were collected over a period of 1 week, for PCR assay and liquid culture by Mycobacterial Growth Indicator Tube. Samples were preferably to be taken before initiation of tuberculosis treatment, but samples that were collected up to 4 weeks after treatment initiation were accepted (case definitions 3 and 4 in Table 1). Diagnostic and treatment decisions were made by treating physicians not involved in the trial. HIV retesting and screening for diabetes (glycated hemoglobin) were performed in all participants with confirmed tuberculosis disease. (For more on surveillance activities, see the Supplementary Appendix.)

STATISTICAL ANALYSIS

Using a log-rank test with 80% power and assuming a true vaccine efficacy of 70% (hazard ratio, 30%) and a two-sided 10% significance level, we estimated that 21 cases of pulmonary tuberculosis were required for a fixed-sample design with the assumption of proportional hazards. To obtain 21 cases, assuming a mean yearly attack rate of 0.55% in the control group, 2 years of follow-up for each participant, and an attrition rate of 15% over the 2-year period, we calculated that 3506 participants would need to be enrolled. As specified in the protocol, the primary analysis could occur after 21 cases had been identified or 24 months of follow-up had been completed.

Vaccine efficacy was analyzed in the according-to-protocol efficacy cohort, with the use of Cox proportional-hazards regression models (vaccine efficacy=1−hazard ratio) with 90% confidence intervals and P values for Wald tests. Descriptive post hoc 95% confidence intervals are also provided. The primary end point was met if the lower limit of the two-sided 90% confidence interval for vaccine efficacy against bacteriologically confirmed pulmonary tuberculosis (first case definition) was more than 0%. If the primary end point was met, the first secondary end point (vaccine efficacy for the second case definition) was to be analyzed according to the same success criterion. A preplanned exploratory analysis compared the effect of six prespecified covariates (giving 14 subgroups) on vaccine efficacy (interpretation should be performed cautiously, because the risk of having at least one false significant result ranges from 51 to 77%).

The total vaccinated cohort (all participants who received at least one dose of M72/AS01$_E$ or placebo) was used to assess safety. Analysis of immunogenicity was performed on the according-to-protocol immunogenicity cohort for the subgroup. Statistical analyses were performed with SAS software, version 9.2 or higher, on the SAS Drug Development system.

RESULTS

TRIAL POPULATION

Of 3575 participants who underwent randomization, 3573 received at least one dose of M72/AS01$_E$ or placebo from August 2014 through November 2015, and 3330 received both doses. The mean (±SD) age of the participants was 28.9±8.3 years; 43% were women. The trial groups were balanced in terms of prespecified demographic characteristics (Table S2 in the Supplementary Appendix).

VACCINE EFFICACY

There were 3283 participants included in the according-to-protocol efficacy analysis (Fig. 1). A total of 10 cases of active pulmonary tuberculosis in the vaccine group and 22 cases in the placebo group met the primary case definition after a mean follow-up of 2.3±0.4 years (Table 2). The incidence of pulmonary tuberculosis (first case definition) per 100 person-years was 0.3 in the M72/AS01$_E$ group and 0.6 in the placebo group, with an overall vaccine efficacy of 54.0% (90% confidence interval [CI], 13.9 to 75.4; 95% CI, 2.9 to 78.2; P=0.04). An analysis that used a Cox

regression model with adjustment for country, sex, diabetes (yes or no), age (≤25 or >25 years), current smoking status (yes or no), and previous BCG vaccination (yes, no, or unknown) gave nearly identical results. Vaccine efficacy for the second case definition (secondary end point) was 58.3% (90% CI, 12.8 to 80.1; 95% CI, −0.5 to 82.7; P=0.05), and vaccine efficacy ranged from 27.7% to 36.4% for protocol-defined case definitions 3 to 5 (Table 2). Kaplan–Meier curves are shown in Figure 2 for the first case definition. Results were similar in the analysis of the total vaccinated efficacy cohort. The incidence of pulmonary tuberculosis (first case definition) per 100 person-years in the total vaccinated cohort was 0.2 in the M72/AS01$_E$ group and 0.5 in the placebo group, with overall vaccine efficacy of 57.0% (90% CI, 19.9 to 76.9; 95% CI, 9.7 to 79.5) (Table 2).

A planned sensitivity analysis of the first case definition was restricted to participants positive for *M. tuberculosis* on at least two diagnostic tests (culture, PCR assay, or both) performed on the sputa collected (Table S3 in the Supplementary Appendix). This analysis included 5 cases in the M72/AS01$_E$ group and 17 cases in the placebo group; the vaccine efficacy was 70.3% (90% CI, 31.3 to 87.1; 95% CI, 19.4 to 89.0) (Table 2). Piecewise analysis of cases (first case definition) occurring before versus after the median follow-up time (1.12 years) showed a vaccine efficacy of 39.0% (90% CI, −42.5 to 73.9; 95% CI, −67.7 to 77.8) in the first period and 66.5% (90% CI, 13.3 to 87.0; 95% CI, −4.0 to 89.2) in the second period.

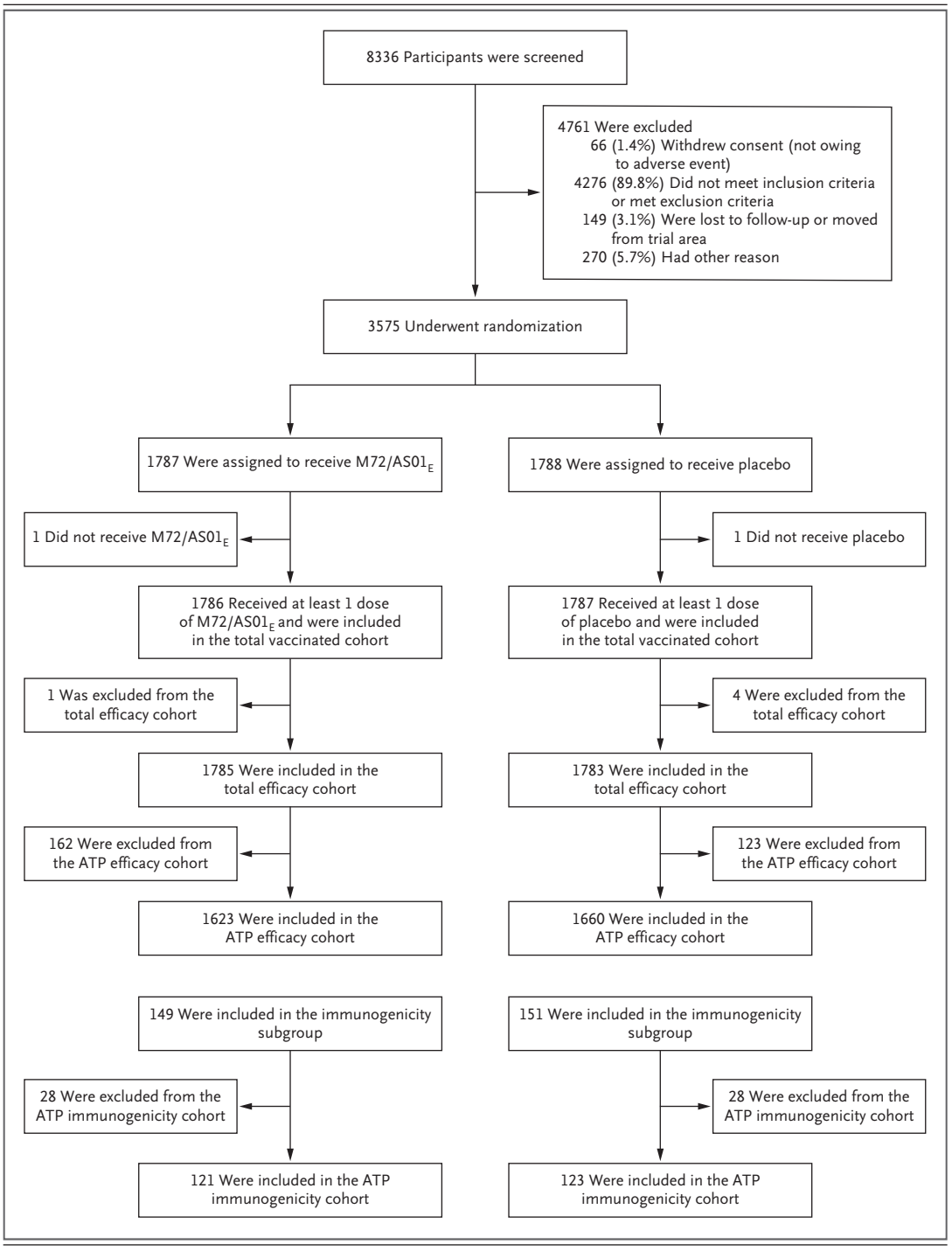
Prespecified subgroup analyses that used case definition 1 showed vaccine efficacy among men of 75.2% (P=0.03) and among women of 27.4% (P=0.52), and vaccine efficacy among participants 25 years or age or younger of 84.4% (P=0.01) and among those older than 25 years of age of 10.2% (P=0.82) (Table 3). A post hoc hierarchical test was performed to assess the interaction between trial group and sex (P=0.31) and between trial group and age (P=0.07) in the complete model containing all main effects as well as the two interaction terms (Table S4 in the Supplementary Appendix).

REACTOGENICITY AND SAFETY

The percentage of participants who had at least one serious adverse event within 6 months after the last dose of either M72/AS01$_E$ or placebo was similar in the two groups (1.6% in the M72/AS01$_E$ group and 1.8% in the placebo group) (Table 4). One serious adverse event in each group was considered to be related to the trial regimen by trial investigators (pyrexia and hypertensive encephalopathy, with blinding to trial-group assignment still in effect). Potential immune-mediated diseases were reported by 2 participants in the M72/AS01$_E$ group and 5 in the placebo group. There were 24 deaths (14 trauma-related) during the trial, with 7 in the M72/AS01$_E$ group (6 trauma-related) and 17 in the placebo group (8 trauma-related) (Table 4). No death was considered to be related to the trial regimen. One participant died of pneumonia, for whom there was also a suspicion of intestinal tuberculosis, but this latter diagnosis was not confirmed. Neither M72/AS01$_E$ nor placebo substantially affected hematologic or biochemical findings (Fig. S1 in the Supplementary Appendix). A post hoc analysis showed 33 pregnancies among 1529 women who received M72/AS01$_E$ or placebo, of which 28 resulted in delivery of a healthy infant. There were 3 ectopic pregnancies as well as one spontaneous abortion, and 1 pregnant woman was lost to follow-up. No birth defects were noted. Regular review by the independent data monitoring committee of unblinded safety data resulted in recommendations to continue the trial unchanged.

There were more unsolicited reports of adverse events in the M72/AS01$_E$ group (67.4%) than in the placebo group (45.4%). The excess was driven by injection-site reactions and influenza-like symptoms (Table S5 in the Supplementary Appendix). Swelling reactions larger than 100 mm in diameter were reported by 53 participants (3.0%) in the M72/AS01$_E$ group and by 1 participant in the placebo group. The median duration of these large swelling reactions was 4 days.

In the immunogenicity subgroup, local and systemic solicited symptoms were reported more frequently by M72/AS01$_E$ recipients than by placebo recipients (Table S6 in the Supplementary Appendix). Among local solicited symptoms, pain was the most frequently reported (81.8% of M72/AS01$_E$ recipients and 34.4% of placebo recipients, with 24.3% and 3.3%, respectively, reporting grade 3 pain). Redness and swelling were uncommon in both groups. Fatigue, headache, malaise, or myalgia was reported by 58.1 to 68.9% of M72/AS01$_E$ recipients and 26.5 to 47.0% of placebo recipients. Fever higher than 38.0°C was reported by 18.9%



and 6.6%, respectively. Fever higher than 39.5°C was reported by 4.1% and 1.3%, respectively.

The immunogenicity results indicate that 100% of the participants in the M72/AS01$_E$ group had seroconversion at month 2 and 99% were seropositive at month 12 (Fig. S2 in the Supplementary Appendix).

DISCUSSION

There is no tuberculosis vaccine recommended for use in *M. tuberculosis*–infected adults, who represent a reservoir of potential cases of active tuberculosis. Here, we found that protection against tuberculosis disease may be achieved by vaccina-

Figure 1 (facing page). Screening and Randomization.
Of the 5 participants who were excluded from the total efficacy cohort, 2 were found to have active tuberculosis at trial entry and 3 had a history of active tuberculosis; blinding to trial-group assignment remains in effect. A total of 285 participants (blinding to trial-group assignment remains in effect) were excluded from the according-to-protocol (ATP) efficacy cohort for the following reasons: administration of vaccine forbidden in the protocol (19 participants), randomization error (2), randomization code broken at the investigator site (1), trial regimen not administered according to the protocol (3), participant did not receive two doses of the trial regimen (236), participant did not enter the efficacy evaluation period 1 month after dose 2 (11), active tuberculosis (any case definition) diagnosed up to 1 month after dose 2 (1), administration of medication forbidden by the protocol (2), nonadherence to the trial-regimen schedule (3), and participant did not meet inclusion criteria or met exclusion criteria (7). A total of 56 participants (blinding to trial-group assignment remains in effect) were excluded from the ATP immunogenicity cohort for the following reasons: administration of vaccine forbidden in the protocol (4 participants), sputum positive for *Mycobacterium tuberculosis* at baseline (1), participant did not meet inclusion criteria or met exclusion criteria (1), concomitant infection (active tuberculosis) that was related to the vaccine and that may influence immune response (1), concomitant infection (participant became HIV-infected) that was not related to the vaccine and that may influence immune response (7), nonadherence to the trial-regimen schedule (3), nonadherence to the blood-sampling schedule (9), essential serologic data missing (all post-vaccination time points at month 2 and month 12 missing) (15), and participant did not receive two doses of the trial regimen (15).

tion of *M. tuberculosis*–infected adults with an adjuvanted subunit vaccine containing two *M. tuberculosis* proteins. The finding of efficacy for the primary end point was supported by the sensitivity analysis and by the analysis of the second case definition. The analyses of less stringent case definitions 3 to 5 did not show significant differences between the M72/AS01$_E$ group and the placebo group. The results with respect to the safety and reactogenicity profile are consistent with those observed previously. Antibody responses were in the same range as observed previously in M72/AS01$_E$-vaccinated adults living in regions in which tuberculosis is endemic.[9,10]

Because the trial included only *M. tuberculosis*–infected persons, it is not possible to determine the extent to which *M. tuberculosis* infection influences vaccine efficacy. In previous tuberculosis efficacy trials, the viral-vectored candidate vaccine MVA85A showed no additional protection beyond that provided by the BCG vaccine in infants not infected with *M. tuberculosis*[24]; multiple doses of inactivated *M. vaccae (obuense)* that were administered to HIV-infected adults reduced the risk of definite tuberculosis, which was a secondary end point, by 39%, with no effect modification according to baseline *M. tuberculosis* infection status.[25] A global tuberculosis vaccination strategy would ideally target both *M. tuberculosis*–infected and uninfected adolescents and adults.[4] Our findings in *M. tuberculosis*–infected adults complement those of a recent trial showing 45% efficacy of BCG revaccination for protection of adolescents not infected with *M. tuberculosis* against sustained QFT seroconversion.[26] Our results suggest further evaluation of M72/AS01$_E$ as a possible vaccination strategy against tuberculosis.

Recent research suggests that progression from latent *M. tuberculosis* infection to active tuberculosis is not a single definitive event but rather a transition through a spectrum of inflammatory and infected states that reflect the activity of individual granulomas.[27] Clinically, this spectrum results in heterogeneous disease states within and between persons. In this trial, participants with clinical suspicion of tuberculosis underwent diagnostic investigation. Approximately one third of confirmed cases of pulmonary tuberculosis were confirmed by a single test of the six performed (either culture or PCR assay). "Single positive" cases were evenly distributed between the vaccine and placebo groups and became positive by culture (7 cases) after an unusually long period or by PCR assay (3 cases) after an unusually high number of amplification cycles. We hypothesize that active surveillance of trial participants detected pulmonary tuberculosis with a low bacterial load, which would be consistent with early stages of disease or reinfection. Three (of 10) "single positive" participants (with blinding as to trial-group assignment) did not receive tuberculosis treatment and remained well, which suggests successful immune control and lack of disease progression.

The sensitivity analysis suggested higher vaccine efficacy among participants with at least two positive tests, which would be consistent with a higher bacterial load. Piecewise and time-to-event analyses did not show significant vaccine efficacy during year 1. We hypothesize that this may be because at least some persons in whom active tuberculosis developed during this time already had incipient tuberculosis at baseline, against which the vaccine would not be expected to have

Table 2. Vaccine Efficacy of M72/AS01$_E$ versus Placebo for Each Case Definition of Pulmonary TB.*

Cohort and Case Definition	M72/AS01$_E$			Placebo			Vaccine Efficacy		
	No. of Participants†	Person-yr of Follow-up	Rate per 100 Person-yr (90% CI)	No. of Participants†	Person-yr of Follow-up	Rate per 100 Person-yr (90% CI)	% (90% CI)	% (95% CI)	P Value‡
According-to-protocol efficacy cohort									
First definition	10	3707.03	0.3 (0.2 to 0.5)	22	3747.43	0.6 (0.4 to 0.8)	54.0 (13.9 to 75.4)	54.0 (2.9 to 78.2)	0.04
Sensitivity analysis	5	3709.42	0.1 (0.1 to 0.3)	17	3751.23	0.5 (0.3 to 0.7)	70.3 (31.3 to 87.1)	70.3 (19.4 to 89.0)	
Second definition	7	3709.42	0.2 (0.1 to 0.4)	17	3751.23	0.5 (0.3 to 0.7)	58.3 (12.8 to 80.1)	58.3 (–0.5 to 82.7)	0.05
Third definition	16	3707.03	0.4 (0.3 to 0.7)	25	3747.43	0.7 (0.5 to 0.9)	35.3 (–9.5 to 61.8)	35.3 (–21.2 to 65.5)	
Fourth definition	17	3707.03	0.5 (0.3 to 0.7)	27	3747.43	0.7 (0.5 to 1.0)	36.4 (–5.9 to 61.8)	36.4 (–16.8 to 65.3)	
Fifth definition	21	3711.87	0.6 (0.4 to 0.8)	30	3753.43	0.8 (0.6 to 1.1)	29.2 (–13.1 to 55.7)	29.2 (–23.7 to 59.5)	
Modified fifth definition	20	3711.87	0.5 (0.4 to 0.8)	28	3753.03	0.7 (0.5 to 1.0)	27.7 (–17.0 to 55.3)	27.7 (–28.3 to 59.3)	
Total vaccinated efficacy cohort									
First definition	10	4301.70	0.2 (0.1 to 0.4)	23	4253.72	0.5 (0.4 to 0.8)	57.0 (19.9 to 76.9)	57.0 (9.7 to 79.5)	0.03
Sensitivity analysis	5	4304.09	0.1 (0.1 to 0.2)	17	4258.75	0.4 (0.3 to 0.6)	70.9 (32.9 to 87.4)	70.9 (21.2 to 89.3)	
Second definition	7	4304.09	0.2 (0.1 to 0.3)	17	4258.75	0.4 (0.3 to 0.6)	59.3 (14.8 to 80.5)	59.3 (1.8 to 83.1)	0.046
Third definition	17	4301.70	0.4 (0.3 to 0.6)	26	4253.72	0.6 (0.4 to 0.8)	35.4 (–7.9 to 61.3)	35.4 (–19.1 to 64.9)	
Fourth definition	18	4301.70	0.4 (0.3 to 0.6)	28	4253.72	0.7 (0.5 to 0.9)	36.5 (–4.4 to 61.3)	36.5 (–14.9 to 64.9)	
Fifth definition	23	4306.54	0.5 (0.4 to 0.8)	30	4260.96	0.7 (0.5 to 1.0)	24.1 (–19.7 to 51.9)	24.1 (–30.6 to 55.9)	
Modified fifth definition	22	4306.54	0.5 (0.4 to 0.7)	28	4260.55	0.7 (0.5 to 0.9)	22.2 (–24.2 to 51.3)	22.2 (–35.9 to 55.5)	

* The analysis was performed with an unadjusted Cox regression model. The according-to-protocol efficacy cohort included 1623 participants who received M72/AS01$_E$ and 1660 who received placebo. The total vaccinated efficacy cohort included 1785 participants who received M72/AS01$_E$ and 1783 who received placebo. Follow-up started 30 days after dose 2 for the analysis of the according-to-protocol cohort and from the day of dose 1 for the analysis of the total vaccinated efficacy cohort and ended for both analyses at the first occurrence of pulmonary TB (for participants meeting a case definition) or at either the end of the participant's follow-up or the date of data lock, whichever came first (for participants not meeting a case definition). CI denotes confidence interval.

† Shown is the number of participants who met the case definition.

‡ Shown are two-sided P values from a Cox regression model.

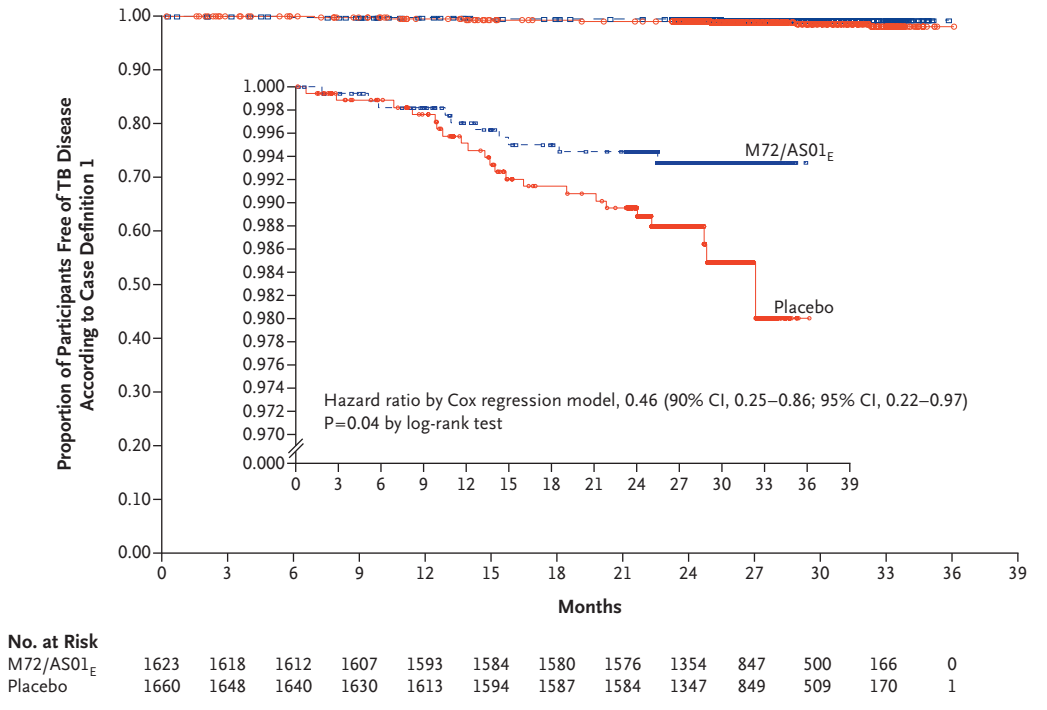


Figure 2. Kaplan–Meier Estimate of Definite Pulmonary Tuberculosis (TB) Disease Not Associated with HIV Infection (First Case Definition).

The analysis was conducted in the according-to-protocol efficacy cohort. The time shown is the time from the beginning of follow-up (i.e., 30 days after dose 2). The inset shows the same data on an enlarged y axis. The decreased number at risk after 24 months reflects the participants for whom follow-up after this time point had not occurred at the date of data lock.

an effect, or that the trial did not have power to show a difference in the first year, or that this was a chance finding. Although we made efforts to exclude participants with active tuberculosis at screening (single PCR test on one sputum specimen), a limitation of the trial was that we could not rule out that early active cases were missed, given the frequently low bacillary load and sporadic nature of bacillary shedding in early stages of tuberculosis disease.[28]

Unexpectedly, we observed a higher point estimate for vaccine efficacy in men than in women (attack rate in the placebo group, 0.6 per 100 person-years for men and women) and in participants 25 years of age or younger than in those older than 25 years of age (attack rate in the placebo group, 0.8 and 0.4 per 100 person-years, respectively). A post hoc demographic analysis showed an imbalance in sex among participants 25 years of age or younger (66% men and 34% women), whereas the older age group was well-balanced, which suggests that the apparent difference observed according to sex was confounded by the effect of age and is probably an artifact. In addition, in a post hoc interaction test, vaccine efficacy did not seem to differ significantly according to sex (P=0.31), whereas efficacy tended to be heterogeneous across age groups (P=0.07 in a hierarchical model containing both interactions). Interpretation of all post hoc and exploratory subgroup analyses should be performed cautiously, because the trial was not powered to detect differences between subgroups, and multiple comparisons were not accounted for.

Age could potentially affect vaccine efficacy through a differential vaccine effect according to the time since primary *M. tuberculosis* infection or BCG priming.[29] We hypothesize that those with less-recent primary infection are more likely to have the infection under immune-system control, with little additional benefit conveyed by vaccination. Increasing age is associated with increased

Table 3. Vaccine Efficacy against Definite Pulmonary TB Disease Not Associated with HIV Infection (Case Definition 1) for Each Covariate and Overall.*

Covariate and Group	No./Total No.†	Person-yr of Follow-up	Rate per 100 Person-yr (90% CI)	Vaccine Efficacy	
				% (90% CI)	% (95% CI)
Overall					
M72/AS01$_E$	10/1623	3707.03	0.3 (0.2 to 0.5)	54.0 (13.9 to 75.4)	54.0 (2.9 to 78.2)
Placebo	22/1660	3747.43	0.6 (0.4 to 0.8)		
Diabetes					
No					
M72/AS01$_E$	10/1615	3688.14	0.3 (0.2 to 0.5)	53.9 (13.8 to 75.4)	53.9 (2.8 to 78.2)
Placebo	22/1655	3735.22	0.6 (0.4 to 0.8)		
Yes					
M72/AS01$_E$	0/7	16.29	0	0	0
Placebo	0/5	12.21	0		
Sex					
Female					
M72/AS01$_E$	7/679	1572.39	0.4 (0.2 to 0.8)	27.4 (–63.4 to 67.7)	27.4 (–90.8 to 72.4)
Placebo	10/708	1627.29	0.6 (0.4 to 1.0)		
Male					
M72/AS01$_E$	3/944	2134.63	0.1 (0.1 to 0.4)	75.2 (28.3 to 91.4)	75.2 (12.2 to 93.0)
Placebo	12/952	2120.13	0.6 (0.4 to 0.9)		
Country					
Kenya					
M72/AS01$_E$	2/242	549.09	0.4 (0.1 to 1.2)	–101.6 (–1411.7 to 73.1)	–101.6 (–2123.7 to 81.7)
Placebo	1/246	550.84	0.2 (0 to 0.9)		
South Africa					
M72/AS01$_E$	8/1307	3008.71	0.3 (0.1 to 0.5)	59.3 (19.0 to 79.6)	59.3 (7.6 to 82.1)
Placebo	20/1344	3058.97	0.7 (0.5 to 0.9)		
Zambia					
M72/AS01$_E$	0/74	—	—		
Placebo	1/70	—	—		
Current smoker					
No					
M72/AS01$_E$	3/791	1812.82	0.2 (0.1 to 0.4)	56.0 (–36.9 to 85.9)	56.0 (–70.1 to 88.6)
Placebo	7/818	1856.06	0.4 (0.2 to 0.7)		
Yes					
M72/AS01$_E$	7/831	1891.62	0.4 (0.2 to 0.7)	53.3 (0.8 to 78.0)	53.3 (–14.6 to 80.9)
Placebo	15/842	1891.36	0.8 (0.5 to 1.2)		
Age					
≤25 yr					
M72/AS01$_E$	2/705	1599.77	0.1 (0 to 0.4)	84.4 (45.7 to 95.5)	84.4 (31.0 to 96.5)
Placebo	13/724	1616.66	0.8 (0.5 to 1.3)		

Table 3. (Continued.)

Covariate and Group	No./Total No.†	Person-yr of Follow-up	Rate per 100 Person-yr (90% CI)	Vaccine Efficacy	
				% (90% CI)	% (95% CI)
>25 yr					
M72/AS01$_E$	8/918	2107.25	0.4 (0.2 to 0.7)	10.2 (–99.6 to 59.6)	10.2 (–132.7 to 65.4)
Placebo	9/936	2130.77	0.4 (0.2 to 0.7)		
BCG vaccination‡					
No					
M72/AS01$_E$	X/136§	—	—		
Placebo	X/149§	—	—		
Yes					
M72/AS01$_E$	8/1243	2823.92	0.3 (0.2 to 0.5)	55.8 (11.0 to 78.0)	55.8 (–1.8 to 80.8)
Placebo	18/1247	2808.34	0.6 (0.4 to 0.9)		
Unknown					
M72/AS01$_E$	1/243	555.68	0.2 (0 to 0.9)	73.1 (–69.1 to 95.7)	73.1 (–140.5 to 97.0)
Placebo	4/264	591.74	0.7 (0.3 to 1.5)		

* The analysis was conducted with an unadjusted Cox regression model in the according-to-protocol efficacy cohort.
† Shown is the number of participants meeting the first case definition and the total number of participants.
‡ BCG (bacille Calmette–Guérin) vaccination indicates documentation of previous BCG vaccination or the presence of a BCG scar.
§ A total of 136 participants in the M72/AS01$_E$ group and 149 in the placebo group had no previous BCG vaccination and no BCG scar. Of these 285 participants, 1 met the first case definition, and blinding to trial-group assignment remains in effect.

probability of more remote infection, according to several studies[30-34] and screening data from the current trial, in which 55.1 to 66.6% of the screened persons were already infected with *M. tuberculosis*.[31] Alternatively, the circumstances that lead to reactivation may be less amenable to immunologic control by booster vaccination further from primary BCG vaccination or initial *M. tuberculosis* infection, and therefore the benefits of vaccination may be more limited. Given the age of the trial population, immune senescence is unlikely to have affected vaccine efficacy.

In our trial, PCR assay had sensitivity of 80% as compared with culture (Table S8 in the Supplementary Appendix), a finding consistent with more events meeting the first case definition than the second. Future trials of vaccine efficacy should therefore use automated liquid culture in addition to PCR assay to maximize case detection. Tuberculosis treatment of adult drug-sensitive pulmonary tuberculosis leads to negative sputum culture and PCR assay at 8 weeks in some participants[35]; therefore, case definitions 3 and 4 probably underestimate the incidence of tuberculosis.

Strengths of the trial were the inclusion of a large, well-defined cohort, exclusion of active tuberculosis disease at baseline, statistical power to address the primary end point, and the use of alternative case definitions for the efficacy end point that reflect applicability in the real world. Finally, 99% of the participants consented to biobanking of blood samples obtained before and after administration of the trial regimen. These samples offer the opportunity to discover potential immune correlates of vaccine-mediated protection against tuberculosis, which, if confirmed, will be useful to reduce the size of future efficacy trials (ClinicalTrials.gov number, NCT02097095). (Fig. S3 in the Supplementary Appendix elaborates on the clinical relevance of the proof-of-concept trial in a form that could be shared with patients by health care professionals.)

In conclusion, we found that the incidence of pulmonary tuberculosis was significantly lower with M72/AS01$_E$ than with placebo among healthy *M. tuberculosis*–infected, largely BCG-vaccinated, HIV-negative adults. These promising results provide an opportunity to better understand the

Table 4. Summary of Vaccine Safety (Total Vaccinated Cohort).*

Variable	M72/AS01$_E$ (N=1786)		Placebo (N=1787)		Relative Risk (95% CI)
	No. of Participants	% (95% CI)	No. of Participants	% (95% CI)	
30 Days after vaccination					
≥1 Unsolicited symptom	1203	67.4 (65.1–69.5)	812	45.4 (43.1–47.8)	1.48 (1.35–1.62)
≥1 Causally related unsolicited symptom	992	55.5 (53.2–57.9)	371	20.8 (18.9–22.7)	2.68 (2.37–3.02)
≥1 Grade 3 symptom	234	13.1 (11.6–14.8)	124	6.9 (5.8–8.2)	1.89 (1.51–2.37)
≥1 Causally related grade 3 symptom	177	9.9 (8.6–11.4)	27	1.5 (1.0–2.2)	6.56 (4.36–10.23)
≥1 Serious adverse event†	10	0.6 (0.3–1.0)	17	1.0 (0.6–1.5)	
≥1 Causally related serious adverse event‡	1	0.1 (0–0.3)	1	0.1 (0–0.3)	
Within 6 mo after vaccination					
≥1 Serious adverse event§	29	1.6 (1.1–2.3)	33	1.8 (1.3–2.6)	
≥1 Causally related serious adverse event‡	1	0.1 (0–0.3)	1	0.1 (0–0.3)	
Potential immune-mediated disease¶	2	0.1 (0–0.4)	5	0.3 (0.1–0.7)	
Entire trial period					
Death‖	7	0.4 (0.2–0.8)	17	1.0 (0.6–1.5)	
Death by injury**	6	—	8	—	

* The causal relationship between the trial regimen and the symptom or serious adverse event was determined by the site investigators.
† Serious adverse events included hypochromic anemia, cardiac disorder, ventricular tachycardia, gastric ulcer, pyrexia, acute HIV infection, cellulitis, lymph-node tuberculosis, malaria, pelvic inflammatory disease, pneumonia, tuberculosis, gunshot wound, head injury, limb injury, traumatic pneumothorax, soft-tissue injury, traumatic hemothorax, wound hematoma, hypertensive encephalopathy, seizure, depression, schizophrenia, acute kidney injury, uterine polyp, and hypertension. Blinding to trial-group assignment remains in effect.
‡ Causally related serious adverse events included pyrexia and hypertensive encephalopathy, with blinding to trial-group assignment still in effect.
§ For details on serious adverse events, see Table S7 in the Supplementary Appendix.
¶ Cases of potential immune-mediated disease included two cases of optic neuritis and one case each of immune thrombocytopenic purpura, Basedow (Graves') disease, gout, erythema multiforme, and morbilliform rash. Blinding to trial-group assignment remains in effect.
‖ In addition to the 14 deaths for which a coding of death from injury was applied, there were 3 cases of death from unknown cause or sudden death and 1 death each from cardiac disorder, hepatic cirrhosis and hepatic encephalopathy, acute HIV infection, pneumonia and suspicion of gastrointestinal tuberculosis, stroke, completed suicide, and dyspnea (drug overdose). For these 10 deaths, blinding to trial-group assignment remains in effect.
** Types of injury included gunshot, stab wound, road traffic accident, and burn.

mechanisms by which this vaccine may confer protection against tuberculosis and support its further evaluation.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

APPENDIX

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